ASSESSING CONTAMINANT SENSITIVITY OF CAPE FEAR SHINER AND SPOTFIN CHUB
Interim Report - MAY, 1999

by

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NOTICE

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INTRODUCTION

The spotfin chub (*Hybopsis monacha*), once widely distributed in the Tennessee River basin from Alabama to Virginia, is now restricted to four river systems in the Tennessee River valley. One of the best remaining populations of this species occurs in the Little Tennessee River, Swain and Macon counties, North Carolina. The spotfin chub population in the Little Tennessee River is currently stable, but this area is threatened by prospects of residential development. Habitat requirements for clear water over gravel, boulders, or bedrock indicates a sensitivity to nonpoint source pollution by sediment. Consequently, poor land use practices associated with forestry, development, mining, and agriculture are thought to have impacted this species (USFWS 1992). Habitat alteration including impounding rivers for power generation have also affected the spotfin chub. While pollution from industrial, agricultural, and domestic sources has been implicated in the species' demise, the spotfin chub's sensitivity to chemical contamination can only be approximated since it has not been tested.

The Cape Fear shiner (*Notropis mekistocholas*) is known from four small populations in the Cape Fear drainage in Randolph, Moore, Lee, Harnett, and Chatham Counties, North Carolina. Total numbers are unknown, but all populations appear to be small. Constituent elements of the species' habitat include clean streams with gravel, cobble, and boulder substrates in pools, riffles, and shallow runs. They are also found in slackwater areas with large rock outcrops and in side channels and pools with good water quality and relatively low silt loads (USFWS 1992). Consequently, this species is also sensitive to nonpoint source pollution by sediment. The Cape Fear shiner may always have existed in low numbers; however, dam construction and the deterioration of water quality are cited as likely factors in the species'

decline (USFWS 1992). As was the case for the spotfin chub, the Cape Fear shiners sensitivity to chemical contamination can only be approximated since it has not been tested.

In order to evaluate the impact of a contaminant release into the environment, standardized toxicity tests are conducted using standard test organisms as surrogates for other species (EPA 1982). Inherent in these programs is the assumption that the test species used for toxicity assessments are predictive of other species. Surrogate species are typically organisms that are easily tested using standardized methods. However, these species may not be representative of all species. The wide use of pesticides and other commercial chemicals invariably poses a risk to aquatic species in decline since, by definition, their distribution is limited and further adverse effects on these populations could lead to extinction. Species may be under protected, or unnecessary regulatory programs may be implemented, if the sensitivity of these species is not evaluated. The following research project provides information for assessing contaminant sensitivity of the Cape fear shiner and spotfin chub. By identifying the sensitivity of these species to contaminant exposures, appropriate regulatory procedures can be implemented for their protection. Acute toxicity tests (96-h LC50) were conducted with Cape Fear shiner and spotfin chub using five chemicals having different toxicological modes of action. Chemicals tested individually included carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin. These chemicals have been tested in cooperative research conducted between the EPA (1995), New York Department of Environmental Conservation (1999), and USGS for the same five chemicals with rainbow trout, fathead minnows and 13 different threatened and endangered species - Apache trout, Lahontan cutthroat trout, greenback cutthroat trout, bonytail chub, Colorado squawfish, razorback sucker, fountain darter, greenthroat darter, shovelnose sturgeon,

gila topminnow, boreal toad, Atlantic sturgeon, and shortnose sturgeon. In that research, similar test conditions were used (static acute toxicity tests, reconstituted ASTM hard water and 60% dilution series) with test temperatures appropriate for the species and selected from the series identified by ASTM (1998).

MATERIALS AND METHODS

Test organisms

Spotfin chub and Cape Fear shiner were obtained as fry from Conservation Fisheries, Knoxville, TN. Fish were held in well water (alkalinity 258 mg/L as $CaCO_3$, hardness 286 mg/L as $CaCO_3$, pH 7.8, 18°C) at the Columbia Environmental Research Center (CERC, Columbia, MO) and cultured until appropriate size (weight) for testing.

Before the start of a toxicity test, organisms were acclimated for a total of 96 h (EPA 1975, ASTM 1998). For the first 48 h, organisms were acclimated to the test water and temperature. The test organisms were then moved to other containers and held for an additional 48 h at the test temperature in 100% test water. Organisms were not fed during the 48 h of holding in 100% test water.

Chemicals

The chemicals used in testing were carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin (Table 1). Chemicals were selected to represent different classes of chemical and modes of toxic action. Organic chemical stock solutions were prepared by dissolving the chemical in reagent grade acetone, whereas stock solutions for copper were prepared by dissolving copper in deionized water. The maximum acetone concentration in any test container was 0.05 mL/L. Analytical results of stock concentrations will be included in the final report.

Toxicity tests

Static acute toxicity tests were conducted in basic accordance with procedures described in EPA (1975) and ASTM (1998). Exposures were conducted in 19.6 L glass jars containing 15 L of test solution. Test water was reconstituted hard water (alkalinity 110 to120 mg/L as $CaCO_3$, hardness 160 to180 mg/L as $CaCO_3$; ASTM 1998). Tests were conducted under ambient lighting.

The exposure series consisted of six concentrations with a 60% dilution series tested in either duplicate or triplicate, depending on availability of organisms. When a solvent was used, both a solvent control (0.05 mL/L) and a dilution water control were included for each species. Individual test series were randomly assigned to a waterbath and location within a waterbath (complete block design).

Fishes were counted into two groups (5 organisms per group) and pooled for each exposure replicate (10 organisms/replicate). All tests with spotfin chub were conducted concurrently. There was an insufficient number of Cape Fear shiner to conduct all five chemical exposures at one time. Therefore, Cape Fear shiners were tested in two groups. In test 1, exposures included carbaryl and permethrin. In test 2, exposures were conducted with copper, 4-nonylphenol, and pentachlorophenol.

Mortality was the endpoint measured at 6, 12, 24, 48, 72, and 96 h of exposure and was defined as the lack of movement for a 5-s observation with the unaided eye. Dead animals were removed at each observational time. The study design is summarized in Table 2.

Water quality

Alkalinity, hardness, and pH were measured on each batch of reconstituted water before

the start of the exposures. The pH was measured on the control, low, medium, and high exposure concentrations at 0 h and in those same treatments if organisms survived to 96 h of exposure. Dissolved oxygen was measured on the control, low, medium, and high exposure concentrations at 0 h and in those same treatments if organisms survived to 48 and 96 h of exposure. A summary of the water quality will be included in the final report.

Statistical analysis

The LC50 and 95% confidence interval for each test was usually calculated using probit analysis. However, when probit analysis was not appropriate (i.e., less than two partial mortalities), LC50s and confidence intervals were calculated using moving average or a non-linear interpolative procedure (Stephan 1977). All LC50s and confidence intervals were determined using nominal concentrations.

PRELIMINARY RESULTS AND DISCUSSION

Toxicity results

There was 100% survival at 96 h of exposure for the Cape Fear shiner and the spotfin chub in both the dilution water and solvent (acetone) controls.

Tables 3 to 7 summarize 96-h LC50s for all five chemicals and each species. In general, at 96 h of exposure, permethrin was the most toxic compound and carbaryl was the least toxic compound. These results were similar to those reported in previous studies (EPA 1995, New York 1999) with these five chemicals. The two phenolic compounds (4-nonylphenol and pentachlorophenol) and copper had LC50s in a similar range of concentrations.

For the following discussion we have included data from the present study and data generated in previous cooperative research conducted between the EPA (1995), New York

Department of Environmental Conservation (1999), and USGS for the same five chemicals with rainbow trout, fathead minnows and 13 different threatened and endangered species - Apache trout, Lahontan cutthroat trout, greenback cutthroat trout, bonytail chub, Colorado squawfish, razorback sucker, fountain darter, greenthroat darter, shovelnose sturgeon, gila topminnow, boreal toad, Atlantic sturgeon, and shortnose sturgeon. In that research, similar test conditions were used (static acute toxicity tests, reconstituted ASTM hard water and 60% dilution series) with test temperatures appropriate for the species and selected from the series specified by ASTM (1998).

In order to evaluate species sensitivity, within a chemical, we ranked 96-h LC50s for each species, including the Cape Fear shiner and spotfin chub, from 1 (high sensitivity - low LC50) up to 16 (low sensitivity - high LC50). Ranks were then averaged across chemicals for each species (Table 8) and then reranked. Overall for the five chemicals, the spotfin chub was the eighth most sensitive species and the Cape Fear shiner was the ninth most sensitive species. Rainbow trout were the seventh most sensitive species while fathead minnows were the least sensitive species tested (overall ranked seventeenth). This finding is important since the Cape Fear shiner and spotfin chub are cyprinids, yet overall they are more similar in contaminant sensitivity to rainbow trout than to the standard surrogate cyprinid, the fathead minnow.

In addition to relative species sensitivity, the magnitude of difference between LC50s is also important. We evaluated the magnitude of difference by determining the frequency that LC50s for either the Cape Fear shiner or spotfin chub were out of the expected range of LC50s that might be found for multiple tests with the same chemical using either rainbow trout or fathead minnows. Utilizing data from a previous study with rainbow trout in which six tests

were conducted for each chemical (EPA 1995), we calculated two factors (lowest 96-h LC50 / mean 96-h LC50; mean 96-h LC50 / highest 96-h LC50) which encompassed the range of LC50s for that chemical. For example, for the six toxicity tests conducted with rainbow trout and carbaryl (EPA 1995), the lowest 96-h LC50 was 1.22 mg/L, the highest 96-h LC50 was 3.11, and the mean 96-h LC50 for the six tests was 1.88 mg/L. Factors calculated for rainbow trout carbaryl exposures were 0.60 and 0.65 with a geometric mean of 0.62. For the five chemicals tested with rainbow trout, the geometric mean factor for all five chemicals was 0.69 with a range of 0.60 (permethrin) to 0.80 (pentachlorophenol). We followed the same procedure for fathead minnows and the five chemicals. Fathead minnows had an geometric mean factor for the five chemicals of 0.65 with a range of 0.57 (pentachlorophenol) to 0.73 (permethrin). If a factor of 0.67 is selected as representative of the normal range in LC50s for either rainbow trout or fathead minnow (expected range = LC50 X 0.67 to LC50 / 0.67) for a specific chemical, then the sensitivities of listed species can be evaluated in terms of how often 96-h LC50s for the listed species differed by more than a factor of 0.67 from the 96-h LC50 for either rainbow trout or fathead minnows.

For all possible comparisons of Cape Fear shiner and spotfin chub to the range of 96-h LC50s that might be expected for fathead minnows (n=10), the two listed cyprinid species have LC50s less than the expected fathead LC50 range for seven of the ten possible comparisons (Tables 3 to 7). When the same comparison is made to rainbow trout (n=10), the two cyprinids have two 96-h LC50s less than the expected range of LC50s for rainbow trout and five LC50s in the range of sensitivities exhibited by rainbow trout. There were only three tests for which the listed cyprinids were less sensitive to contaminant exposure than the rainbow trout.

Collectively, these results indicate that the two cyprinid species may be more similar in contaminant sensitivity to rainbow trout.

A final evaluation would be to determine the difference between the 96-h LC50s of the rainbow trout and Cape Fear shiner and spotfin chub. Within a chemical, we compared the 96-h LC50 for either listed cyprinid to the geometric mean 96-h LC50 for rainbow trout (listed species 96-h LC50 / rainbow trout 96-h LC50). For Cape Fear shiner the factors ranged from 0.7 for 4-nonylphenol to 2.4 for carbaryl with an average factor of 1.4. For spotfin chub the factors ranged from 0.4 for 4-nonylphenol to 1.8 for carbaryl with an average factor of 1.1. The average factor for all ten comparison was 1.2 with a range of 0.4 to 2.4.

Overall, these assessments would indicate that the Cape Fear shiner and the spotfin chub are more sensitive to contaminant exposure than the fathead minnow. If rainbow trout is used as a test species, a species typically used in pesticide registration or water quality criteria derivation, those procedures which protect the rainbow trout would likely be protective of the Cape Fear shiner and spotfin chub. However, if population is at risk and a conservative estimator must be used to estimate LC50s for either of these listed fish species, our data indicates that 0.4 would be a conservative estimator. Finally, if EPA water quality criteria are recalculated by eliminating certain species from the data set, such as rainbow trout, then the Cape Fear shiner or spotfin chub species might not be adequately protected.

Additional research

This project includes a second research objective. That objective is to evaluate the sensitivity of Cape Fear shiner and spotfin chub using effluent toxicity testing procedures and comparing the responses of those fish to fathead minnows and *Ceriodaphnia dubia*. Tests are to

be conducted with 1) a five chemical mix (carbaryl, copper, 4-nonylphenol, pentachlorophenol, permethrin), 2) ammonia, 3) chlorine, 4) diazinon, and 5) copper. All tests will be conducted in ASTM hard water, except copper, which will be tested in both ASTM hard water and a reconstituted water with a hardness of 9 mg/L as $CaCO_3$ (a water quality similar to some of the waters inhabited by the shiner and the chub). All of the studies with the spotfin chub have been completed except for the exposure to diazinon. In that case, we have had difficulties obtaining successful tests, potentially because of volatility of the compound. Additionally, we have had difficulties in obtaining successful effluent toxicity tests with the Cape Fear shiner. In those tests, there have been individual replicates that have had complete mortality. These die-offs are not related to dose response and occur in the controls as well as in low exposure concentrations. Attempts will be made again this year to conduct toxicity tests with the Cape Fear shiner.

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Table 1. Source and percent active ingredient of chemicals used in toxicity tests.

Chemical	Source	Active Ingredient (%)	Use	Mode of Action
Carbaryl	Donated by Rhone-Pôulenc Agricultural Co., Research Triangle Park, NC	99.7	carbamate insecticide	inhibitor of cholinesterase activity
Copper sulfate	Fisher Chemical, St. Louis, MO	25.5	mining, industrial, fungicide	interferes in osmoregulation
4-nonylphenol	Fluka Chemical, New York, NY	85.0	nonylphenol ethoxylate detergents	narcotic and oxidative stressor
Pentachlorophenol	Aldrich Chemical, Milwaukee, WI	99.0	wood preservative, molluscicide	uncoupler of oxidative phosphorylation
Permethrin	Donated by ICI Americas Inc., Richmond, CA	95.2	pyrethroid insecticide	neurotoxin

Table 2. Summary of study design for the comparative toxicity of selected chemicals to listed species.

Test type:	Static acute
Test volume:	Cape Fear shiner - 15 L Spotfin chub - 15 L
Test temperature:	Cape Fear shiner - 17°C Spotfin chub - 17°C
Water Quality:	Reconstituted ASTM hard (alkalinity 110 to 120 mg/L as $CaCO_3$, hardness 160 to 180 mg/L as $CaCO_3$)
Chemicals:	Carbaryl, copper, 4-nonylphenol, pentachlorophenol, permethrin
Dilution series:	60%
Replicates/number of organisms per replicate:	Cape Fear shiner - 3 replicates/10 fish per replicate Spotfin chub - 2 replicates/10 fish per replicate
Average weight:	Cape Fear shiner - test 1 - 0.39 g test 2 - 0.61 g Spotfin chub - 0.17 g
Observations:	Mortality at 6, 12, 24, 48, 72, and 96 h of exposure

Table 3. Acute toxicity of carbaryl (mg/L) to 16 fishes and one amphibian. Data includes the 96-h LC50 and the relative species rank sensitivity. Also included is an assessment to determine if the 96-h LC50 for a particular species is out of the average range of LC50s for either rainbow trout or fathead minnows. A notation of X< > was used if the LC50 for an individual species was less than the expected range of LC50s for either rainbow trout or fathead minnow. If the LC50 for a species was in the expected range for either rainbow trout or fathead minnows, a notation of <X> was used, and if greater than the expected range, a notation of <>X was used. In addition, a factor (RBT LC50 ratio) is calculated which relates the geometric mean LC50 for rainbow trout (n=6, EPA 1995) to the LC50 for all other individual species.

Species	Carbaryl				
	LC50	Rank	RBT <0.67>	RBT LC50 Ratio	FHM <0.67>
Rainbow trout	1.88	5	-	1.0	X< >
Fathead minnow	5.21	14	< >X	2.8	-
Apache trout	1.54	2	<X>	0.8	X< >
Greenback cutthroat trout	1.55	3	<X>	0.8	X< >
Lahontan cutthroat trout	2.25	8	<X>	1.2	X< >
Bonytail chub	3.49	11	< >X	1.9	X< >
Colorado squawfish	3.07	9	< >X	1.6	X< >
Razorback sucker	4.35	12	< >X	2.3	<X>
Fountain darter	2.02	6	<X>	1.1	X< >
Greenthroat darter	2.14	7	<X>	1.1	X< >
Shovelnose sturgeon	nc[1]	-	-	-	-
Gila topminnow	>3.0	nr[2]	-	nc	-
Boreal toad	12.3	15	< >X	6.5	< >X
Shortnose sturgeon	1.81	4	<X>	1.0	X< >
Spotfin chub	3.41	10	< >X	1.8	X< >
Cape Fear shiner	4.51	13	< >X	2.4	<X>
Atlantic sturgeon	<0.8	1	X< >	-	X< >

[1]nc - not calculated
[2]nr - not ranked

Table 4. Acute toxicity of copper (mg/L) to 16 fishes and one amphibian. Data includes the 96-h LC50 and the relative species rank sensitivity. Also included is an assessment to determine if the 96-h LC50 for a particular species is out of the average range of LC50s for either rainbow trout or fathead minnows. A notation of X< > was used if the LC50 for an individual species was less than the expected range of LC50s for either rainbow trout or fathead minnow. If the LC50 for a species was in the expected range for either rainbow trout or fathead minnows, a notation of <X> was used, and if greater than the expected range, a notation of < >X was used. In addition, a factor (RBT LC50 ratio) is calculated which relates the geometric mean LC50 for rainbow trout (n=6, EPA 1995) to the LC50 for all other individual species.

Species	Copper				
	LC50	Rank	RBT <0.67>	RBT LC50 Ratio	FHM <0.67>
Rainbow trout	0.08	5.5	-	1.0	X< >
Fathead minnow	0.47	16	< >X	5.9	-
Apache trout	0.07	3.5	<X>	0.9	X< >
Greenback cutthroat trout	>0.03	nr[2]	-	-	-
Lahontan cutthroat trout	0.07	3.5	<X>	0.9	X< >
Bonytail chub	0.22	12	< >X	2.8	X< >
Colorado squawfish	0.43	15	< >X	5.4	<X>
Razorback sucker	0.27	14	< >X	3.4	X< >
Fountain darter	0.06	1.5	<X>	0.8	X< >
Greenthroat darter	0.26	13	< >X	3.3	X< >
Shovelnose sturgeon	0.16	10.5	< >X	2.0	X< >
Gila topminnow	0.16	10.5	< >X	2.0	X< >
Boreal toad	0.12	9	<X>	1.5	X< >
Shortnose sturgeon	0.08	5.5	<X>	1.0	X< >
Spotfin chub	0.09	7	<X>	1.1	X< >
Cape Fear shiner	0.11	8	<X>	1.4	X< >
Atlantic sturgeon	0.06	1.5	<X>	0.8	X< >

[1]nc - not calculated
[2]nr - not ranked

Table 5. Acute toxicity of 4-nonylphenol (mg/L) to 16 fishes and one amphibian. Data includes the 96-h LC50 and the relative species rank sensitivity. Also included is an assessment to determine if the 96-h LC50 for a particular species is out of the average range of LC50s for either rainbow trout or fathead minnows. A notation of X< > was used if the LC50 for an individual species was less than the expected range of LC50s for either rainbow trout or fathead minnow. If the LC50 for a species was in the expected range for either rainbow trout or fathead minnows, a notation of <X> was used, and if greater than the expected range, a notation of < >X was used. In addition, a factor (RBT LC50 ratio) is calculated which relates the geometric mean LC50 for rainbow trout (n=6, EPA 1995) to the LC50 for all other individual species.

Species	**4-Nonylphenol**				
	LC50	**Rank**	**RBT <0.67>**	**RBT LC50 Ratio**	**FHM <0.67>**
Rainbow trout	0.19	11.5	-	1.0	<X>
Fathead minnow	0.27	15	<X>	1.4	-
Apache trout	0.17	8.5	<X>	0.9	X< >
Greenback cutthroat trout	0.15	7	<X>	0.8	X< >
Lahontan cutthroat trout	0.18	10	<X>	0.9	<X>
Bonytail chub	0.29	16	< >X	1.5	<X>
Colorado squawfish	0.26	14	<X>	1.4	<X>
Razorback sucker	0.17	8.5	<X>	0.9	X< >
Fountain darter	0.11	4	X< >	0.6	X< >
Greenthroat darter	0.19	11.5	<X>	1.0	<X>
Shovelnose sturgeon	<0.13	nr[2]	-	-	X< >
Gila topminnow	0.23	13	<X>	1.2	<X>
Boreal toad	0.12	5	X< >	0.6	X< >
Shortnose sturgeon	0.08	2.5	X< >	0.4	X< >
Spotfin chub	0.08	2.5	X< >	0.4	X< >
Cape Fear shiner	0.14	6	<X>	0.7	X< >
Atlantic sturgeon	0.05	1	X< >	0.3	X< >

[1]nc - not calculated
[2]nr - not ranked

Table 6. Acute toxicity of pentachlorophenol (mg/L) to 16 fishes and one amphibian. Data includes the 96-h LC50 and the relative species rank sensitivity. Also included is an assessment to determine if the 96-h LC50 for a particular species is out of the average range of LC50s for either rainbow trout or fathead minnows. A notation of X< > was used if the LC50 for an individual species was less than the expected range of LC50s for either rainbow trout or fathead minnow. If the LC50 for a species was in the expected range for either rainbow trout or fathead minnows, a notation of <X> was used, and if greater than the expected range, a notation of < >X was used. In addition, a factor (RBT LC50 ratio) is calculated which relates the geometric mean LC50 for rainbow trout (n=6, EPA 1995) to the LC50 for all other individual species.

Species	Pentachlorophenol				
	LC50	Rank	RBT <0.67>	RBT LC50 Ratio	FHM <0.67>
Rainbow trout	0.16	4	-	1.0	<X>
Fathead minnow	0.25	10	< >X	1.6	-
Apache trout	0.11	2.5	<X>	0.7	<X>
Greenback cutthroat trout	>0.01	nr[2]	-	-	-
Lahontan cutthroat trout	0.17	5	<X>	1.1	<X>
Bonytail chub	0.23	8	<X>	1.4	<X>
Colorado squawfish	0.24	9	<X>	1.5	<X>
Razorback sucker	0.28	12	< >X	1.8	<X>
Fountain darter	0.11	2.5	<X>	0.7	X< >
Greenthroat darter	0.18	6	<X>	1.1	<X>
Shovelnose sturgeon	nc[1]	-	-	-	-
Gila topminnow	0.34	13	< >X	2.1	<X>
Boreal toad	0.37	14	< >X	2.3	<X>
Shortnose sturgeon	0.07	1	X< >	0.4	X< >
Spotfin chub	0.26	11	< >X	1.6	<X>
Cape Fear shiner	0.19	7	<X>	1.2	<X>
Atlantic sturgeon	nc	-	-	-	-

[1]nc - not calculated
[2]nr - not ranked

Table 7. Acute toxicity of permethrin (ug/L) to 16 fishes and one amphibian. Data includes the 96-h LC50 and the relative species rank sensitivity. Also included is an assessment to determine if the 96-h LC50 for a particular species is out of the average range of LC50s for either rainbow trout or fathead minnows. A notation of X< > was used if the LC50 for an individual species was less than the expected range of LC50s for either rainbow trout or fathead minnow. If the LC50 for a species was in the expected range for either rainbow trout or fathead minnows, a notation of <X> was used, and if greater than the expected range, a notation of < >X was used. In addition, a factor (RBT LC50 ratio) is calculated which relates the geometric mean LC50 for rainbow trout (n=6, EPA 1995) to the LC50 for all other individual species.

Species	Permethrin				
	LC50	Rank	RBT <0.67>	RBT LC50 Ratio	FHM <0.67>
Rainbow trout	3.31	7	-	1.0	X< >
Fathead minnow	9.38	11	< >X	2.8	-
Apache trout	1.71	5	X< >	0.5	X< >
Greenback cutthroat trout	>1.0	nr	-	-	-
Lahontan cutthroat trout	1.58	3	X< >	0.5	X< >
Bonytail chub	>25.0	13	< >X	-	< >X
Colorado squawfish	24.4	12	< >X	7.4	< >X
Razorback sucker	5.95	10	< >X	1.8	X< >
Fountain darter	3.34	8	<X>	1.0	X< >
Greenthroat darter	2.71	6	<X>	0.8	X< >
Shovelnose sturgeon	nc[1]	nr[2]	-	-	-
Gila topminnow	>10.0	nr	< >X	-	-
Boreal toad	>10.0	nr	< >X	-	-
Shortnose sturgeon	<1.2	1.5	X< >	-	X< >
Spotfin chub	1.70	4	X< >	0.5	X< >
Cape Fear shiner	4.16	9	<X>	1.3	X< >
Atlantic sturgeon	<1.2	1.5	X< >	-	X< >

[1]nc - not calculated
[2]nr - not ranked

Table 8. Summary rank for 16 fishes and one amphibian. The summary rank was calculated by averaging the individual ranks obtained for each species (Tables 4 to 8) within a chemical and then reranking.

Family	Species	Summary rank
Salmonidae	Rainbow trout	7
Cyprinidae	Fathead minnow	17
Salmonidae	Apache trout	3
Salmonidae	Greenback cutthroat trout	5
Salmonidae	Lahontan cutthroat trout	6
Cyprinidae	Bonytail chub	15
Cyprinidae	Colorado squawfish	14
Catostomidae	Razorback sucker	13
Percidae	Fountain darter	4
Percidae	Greenthroat darter	10
Acipenseridae	Shovelnose sturgeon	11
Poeciliidae	Gila topminnow	16
Bufonidae	Boreal toad	12
Acipenseridae	Shortnose sturgeon	2
Cyprinidae	Spotfin chub	8
Cyprinidae	Cape Fear shiner	9
Acipenseridae	Atlantic sturgeon	1